IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Cutitta *et al.*
Application No. 10/571,012
Filed: March 8, 2006
Confirmation No. 4600
For: NON PEPTIDE AGONISTS AND ANTAGONISTS OF ADRENOMEDULLIN AND GASTRIN RELEASING PEPTIDE
Examiner: Anna Pagonakis
Art Unit: 1628
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DECLARATION OF DR. FRANK CUTTITTA UNDER 37 C.F.R. § 1.132

I, Frank Cuttitta, Ph.D., declare as follows:

1. I am an inventor listed on U.S. Patent Application No. 10/571,012, filed March 8, 2006.

2. I hold a Ph.D. in Microbiology/Immunology from the University of Maryland. I am the Director of the NCI Angiogenesis Core Facility in Gaithersburg, Maryland. My *curriculum vitae* is attached as **Exhibit A**. By virtue of my education, training, and professional experience, I am knowledgeable about the biology of gastrin releasing peptide (GRP) and the identification and activity of agonists and antagonists of GRP function.

3. I have read the Office Action dated June 10, 2010 and the Advisory Action dated August 26, 2010.

4. I understand that the Office has rejected the claims of the application for allegedly failing to comply with the enablement requirement. Specifically, the Office alleges that the specification "lacks adequate guidance, direction or discussion to apprise the skilled artisan how

the claimed compound may be used to achieve (1) the inhibition of GRP activity and (2) the disclosed utilities for treating conditions wherein GRP inhibition has been implicated" (Office Action, at page 3).

5. GRP affects cellular function by stimulating the release of the second messenger IP_3 in cells expressing the GRP receptor. In cultured cells, in the absence of exogenously added or endogenously secreted GRP, a GRP-specific pure antagonist will have no effect on GRP-stimulated IP_3 release. GRP-stimulated activity is well known in the art to be associated with many biological processes, including cell proliferation, lung development, food intake, and control of blood pressure (Cuttitta *et al., Nature*, 316:823-826, 1985; Sunday *et al., J Clin Invest,* 102:584-594, 1998; Merali *et al., Neuropeptides*, 33:376-386, 1999; and Ohki-Hamazaki *et al., Nature*, 390:165-169, 1997; each of which is of record in this file).

6. Based on my experience and knowledge, I believe that one of skill in the art would understand from the specification that the claimed compound can be used to inhibit GRP activity.

7. The subject compound is designated in the claims as comprising "a compound of formula XV'." In the specification, and in the related published literature, this compound is also designated compound 77427. Thus, compound 77427 is synonymous with a compound of formula XV'.

8. As described in the specification, compound 77427 was identified as a GRP inhibitor by primary and secondary screens of a small molecule library. The primary screen identified compounds that blocked the interaction between GRP and a GRP-binding antibody that inhibits GRP function (*i.e.* a neutralizing antibody). This screen is set forth schematically in Figure 1A of the specification. The secondary screen tested the ability of the compounds identified in the primary screen to **affect GRP activity in cultured cells expressing the GRP receptor** (*i.e.* test the ability of the compounds that were identified to block the binding of the GRP-binding antibody to stimulate or inhibit GRP activity). Non-small cell lung carcinoma H1299 cells were used in the secondary screen; H1299 cells are known in the art to express the GRP receptor (*see*

Moody *et al.*, *J. Cell. Biochem. Supp.*, 24:247-256, 1996; provided herewith as **Exhibit B**), but these cells do not express significant amounts of GRP (*see* Giaccone, *J. Cancer Res. (Supp.)*, 52:2732s-2736s, 1992; provided herewith as **Exhibit C**). Figure 3 of the specification presents data from the secondary screen. Figure 3A shows that in the absence of any additional GRP or compound (control), relatively little IP_3 release is detectable. This confirms that the H1299 cells secrete little if any GRP into the culture media, and also determines the baseline of IP_3 release (non-GRP stimulated) in the cell cultures. When GRP is added to the culture media, the level of IP_3 released is significantly increased. This confirms that the H1299 cells express the GRP receptor, and that exogenously-added GRP will stimulate IP_3 release. In contrast, when both GRP and compound 77427 are added to the culture media, relatively little IP_3 is released.

9. The results presented in Figure 3 clearly and unequivocally indicate that compound 77427 inhibits the IP_3-stimulating activity of GRP. Moreover, because GRP function is dependent on the ability to stimulate second messenger release, I conclude that these results also indicate that compound 77427 will inhibit all known GRP activities. This conclusion is further supported by additional data provided in the specification.

10. Additional evidence that compound 77427 inhibits GRP activity is provided in Figures 5 and 6, both of which illustrate that compound 77427 inhibits the angiogenesis-stimulating activity of GRP, *in vitro* (Figure 5) and *in vivo* (Figure 6). Figure 5 shows the effect of GRP and compound 77427 on *in vitro* endothelial cell preangiogenic cord formation. In the absence of GRP, relatively few endothelial cell cord structures are formed (top). When GRP is added, abundant endothelial cord structures are observable (middle). When GRP is added together with compound 77427, relatively few cord structures are observed (bottom). Thus, compound 77427 inhibits GRP-stimulated cord formation activity. This and similar results have since been published in Martínez *et al.*, *Oncogene*, 24:4106-4113, 2005 (of record) and Fang *et al.*, *Lymphatic Res. and Biology*, 7: 189-196, 2009 (provided herewith as **Exhibit D**).

11. Figure 6 shows the effects of GRP and the combination of GRP and compound 77427 on blood vessel formation in a directed *in vivo* angiogenesis assay (DIVAA). The DIVAA method and the interpretation of the results in Figure 6 are both described in Example 7

of the specification at page 36, lines 11-23. As described therein, in this assay, silicone tubes with only one end open (angioreactors) were filled with 20 µl extracellular gel matrix (matrigel), either alone or mixed with GRP and/or a GRP inhibitor. After the matrigel solidified, the angioreactors were implanted into the dorsal flanks of athymic nude mice. After 11 days, the mice were injected intravenously with 25 mg/ml FITC-dextran 20 minutes before removing angioreactors. Quantitation of neovascularization in the angioreactors was determined as the amount of fluorescence trapped in the implants and was measured in a HP Spectrophotometer. Thus, greater relative fluorescence units (RFU) shown in Figure 6 indicates more angiogenesis, and less RFU indicates less angiogenesis. As shown in Figure 6, angiogenesis is stimulated in the presence of GRP. This stimulated angiogenesis is inhibited in a dose-dependent manner as the concentration of compound 77427 that is added with GRP is increased.

12. Figure 6 also shows that the inhibitory effects of compound 77427 on GRP-stimulated angiogenesis are similar to the effects of the known GRP inhibitor, antibody 2A11. Both agents affect GRP-stimulated angiogenesis in a dose-dependent manner. As discussed below, antibody 2A11 has been used extensively in my laboratory and by others in experiments to inhibit proliferation of multiple cancer cell types and to treat chronic lung disease in an animal model. Thus, Figure 6 not only demonstrates that compound 77427 inhibits GRP function, but also illustrates that compound 77427 inhibits GRP function in the same way as a known GRP inhibitor that has been previously used to treat GRP-stimulated disease. I understand that the Office questions this conclusion in part because "compound 77427 and compound 2A11 in Figure 6 are administered at significantly different amounts" (Advisory Action, page 3). Compound 77427 and monoclonal antibody 2A11 are different types of GRP-inhibitory molecules, and so one of ordinary skill would not be surprised if different units of concentration were used in their administration and different amounts are administered. In addition, the actual concentrations of compound and antibody used are in fact quite similar. The units of antibody administered in Figure 6 can readily be converted from µg/mL to nM, using 150 kDa (150,000 g/mol) as the recognized approximate molecular weight of an IgG (such as antibody 2A11). In Figure 6, the maximum effective concentration of compound 77427 is **500 nM**. The maximum effective concentration of antibody 2A11 is 100 µg/mL. When converted into nM, this concentration is approximately **670 nM** (100 µg/mL X 150,000 g/mol). Thus, Figure 6

demonstrates that compound 77427 and antibody 2A11 function at comparable maximum effective concentrations (500 nM versus 670 nM).

13. I understand that the Office maintains that compound 77427 cannot be claimed as a GRP inhibitor because the specification generally refers to the compounds identified therein as "modulating" compounds (Office Action, at page 8 and Advisory Action, at page 3). The specification indeed describes both agonists and antagonists of GRP, and these compounds are grouped together under the label "modulating compounds." However, when these compounds are described individually (for example as in Table 1 at pages 18-19 of the specification), the compounds are labeled according to their particular characteristics. Thus, compound 77427 is clearly labeled in Table I as an antagonist of GRP-stimulated second messenger activity. I note that such general and specific labels are commonly used in the art. *See* for example, Martinez *et al. Endocrinology*, 145:3858-3865, 2004 (of record). Similarly, in **Exhibit D**, agonist and antagonist compounds are referred to together as "regulators," but specifically designated as "agonists" or "antagonists" as appropriate when discussed individually.

14. Based on the data provided in the specification, I conclude that the ability of compound 77427 to inhibit GRP activity can be extended to treatment of various conditions where GRP activity has been implicated. As discussed above, Figure 6 of the specification clearly demonstrates that compound 77427 inhibits GRP activity in an analogous manner to GRP neutralizing antibody 2A11. GRP neutralizing antibodies (*e.g.* antibody 2A11), which inhibit GRP activity by blocking GRP binding to its cellular receptor, are well known to the art (*see* for example, Cuttitta *et al., Nature*, 316:823-826, 1985; of record). These antibodies have been used to block GRP activity in many different contexts. For example, GRP neutralizing antibodies have been used to decrease proliferation of several types of cancer cells including lung cancer (*Id.*), pancreatic cancer (Avis *et al., Molecular Carcinogenesis,* 8:214-220, 1993; of record), and squamous cell carcinoma (Lango *et al., Journal of the National Cancer Institute,* 94:375-383, 2002; of record). Another exemplary use of GRP neutralizing antibodies has been to treat chronic lung disease in an animal model of brochopulmonary dysplasia (BPD) (Sunday *et al., The Journal of Clinical Investigation,* 102:584-594, 1998; of record). Because compound 77427 inhibits GRP activity in an analogous manner to GRP neutralizing antibody 2A11, I understand

and could reasonably predict (through sound scientific reasoning) that compound 77427 will provide a therapeutic benefits similar to those previously observed with GRP neutralizing antibodies (such as antibody 2A11).

15. In summary, based on my education, training, and professional experience, I believe that the specification provides sufficient guidance to enable one of skill in the art to practice the invention as claimed.

16. I hereby declare that all statements made herein are of my own knowledge, are true and that all statements made on information and belief are believed to be true. Furthermore, these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of the Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

September 8, 2010
Date

Name Frank Cuttitta, Ph.D.